

Topsmelt *Atherinops affinis* 4-Day Acute Toxicity Test

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of water samples, larval topsmelt are exposed to test solutions for 4 days, after which the percent mortality is determined in each toxicant concentration. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as dissolved oxygen, pH, salinity, ammonia, and temperature.

In this procedure, water samples collected from field stations are divided into replicate beakers in the laboratory. Five randomly selected larval topsmelt are placed into each replicate container. Each beaker is monitored daily for mortality. After a 4-day exposure, survival is counted and recorded to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (US EPA 2002).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

2.1 Culture and Acclimation

- larval fish are obtained from Aquatic Bio Systems (Fort Collins, CO)
- airstones and aeration
- 20- μ m-filtered and 1- μ m-filtered seawater from a uniform source
- brine shrimp *Artemia* cysts for producing *Artemia* nauplii
- smooth glass tubes; 5-mm-bore, 15-cm length, with suction bulbs

2.2 Test Initiation

- 1000 mL clean glass beakers (5 per sample concentration)
- 1000 mL plastic tri-pour reference toxicant test containers
- 1000 mL volumetric flasks and pipettes for reference toxicant dilutions
- Plastic screen tube 150- μ m mesh, 25 cm diameter for fish
- Plastic screen tube 100- μ m mesh for *Artemia*
- Water bath or environmental chamber
- Randomization sheet to arrange and identify test containers
- Data sheets

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- Gloves and appropriate safety gear (see MPSL lab safety manual)
- Sample vials for reference toxicant analysis (new polyethylene 60 mL)
- Graduated pipettes
- Analytical balance
- Plastic squirt bottles

2.3 Water Quality

- Meters and probes for measuring dissolved oxygen, pH, salinity, and ammonia
- Thermometers (glass mercury thermometer and continuously recording thermometer)
- Graduated pipettes and hand pipette pump for water quality sampling
- Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

Dilution water consists of ambient Granite Canyon seawater, filtered to 1 μm , at ambient salinity (33-34‰).

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of five replicate test beakers for each sample concentration. Beakers are arranged randomly, and each receives five randomly selected larval topsmelt. The quality of test fish and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Dissolved oxygen, pH, salinity, temperature and ammonia are measured at the beginning and end of the exposure. New and old dissolved oxygen, pH, temperature and salinity are measured at the 48-hour renewal. Temperature is also measured continuously by a temperature logger. The photoperiod for the test is 16 hours light:8 hours dark, and the temperature is $20 \pm 1^\circ\text{C}$.

4.0 SAMPLE PREPARATION

Because of the 48-hour holding time, tests will generally be initiated on the same day as sample receipt. Label one-liter beakers as indicated on the randomization sheet generated for the test. Determine the salinity of the test solutions. Be sure to stir all samples before measuring salinity, and measure salinity immediately after stirring. Samples salinities below 32‰ are adjusted to 34‰ using hypersaline brine made from frozen seawater or artificial

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salts. Check the pH of brine. If necessary, adjust the brine pH by adding acid or sodium hydroxide until it is between 7.5-8.5.

Using the random number sheet, aliquot 200 mL of sample to beakers. Minimize sample exposure to sunlight (never leave samples in direct sunlight), and schedule loading times to avoid prolonged sample exposure to temperatures above 20°C.

5.0 CONTROLS

5.1 Seawater and Brine Controls

The seawater control consists of 20 µm filtered Granite Canyon seawater. If a brine control is necessary, it should be prepared to contain the same proportion of brine as the lowest salinity sample that was adjusted.

5.2 Reference Toxicant Tests

A reference toxicant test must be conducted concurrently with every test to indicate the sensitivity of the organisms and the suitability of the test methodology. Zinc sulfate (ZnSO_4) should be used as the reference toxicant for topsmelt tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 10,000 µg/L zinc stock solution by adding 0.0440g ZnSO_4 to one liter of distilled water in a volumetric flask. Cap tightly and mix thoroughly. Sample the reference toxicant stock solution at the beginning of the test for chemical verification of the zinc concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N reagent grade nitric acid. Reference toxicant solutions consist of five replicates of the following concentrations: 0, 56, 100, 180, and 320 µg/L. Other concentrations may be added between these if greater precision is desired for quality control chart purposes.

All tests (sample and reference toxicant) must use fish from the same shipment. They must be handled in the same way and delivered to the test containers at the same time.

6.0 TEST INITIATION

6.1 Water Quality Measurements

Before test initiation, measure dissolved oxygen, pH, salinity, and temperature on each concentration of the test and the reference toxicant test. Dissolved oxygen concentration should be 4.0 mg/L. If not, aerate gently until DO increased above 4.0 mg/L.

6.2 Randomized Loading of Topsmelt

Transfer larval topsmelt from their holding tub into test containers using disposable plastic pipets or a fire-polished glass tube. Repeat this process until all beakers are loaded. Place acrylic covers over each set of beakers on a shelf.

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Maintain water temperature ($20 \pm 1^\circ\text{C}$) by sorting animals in the constant temperature room where the test is being held.

7.0 MONITORING THE TOXICITY TEST

7.1 Counting Mysid Mortality and Feeding

Test duration is 4 days. Check all test containers daily, and record the number of dead larvae. Immobile fish that do not respond to a stimulus are considered dead. The stimulus should be a gentle stream of water from a Pasteur pipette. Fish that exhibit any response clearly visible to the naked eye are considered living. Remove dead animals, *Artemia* and other debris. Mysids are fed 40 freshly hatched *Artemia* nauplii per larva twice daily.

7.2 Test Solution Renewal

Because toxicity may change over short time periods in test containers, the test solutions must be renewed after 48 hours. Measure temperature, dissolved oxygen, pH, and salinity on new and old water.

Remove 75% of the original test solution from each container, taking care to avoid losing or damaging fish. This can be accomplished by siphoning with a small-bore (2 to 3 mm) fire-polished glass tube or pipette. Attach the glass tube to clear plastic tubing fitted with a pinch clamp so that the siphon flow can be stopped quickly if necessary to release entrained fish. Siphon tubes must be leached for 24 hours prior to renewal in seawater.

To minimize disturbance to the larval topsmelt, refill the containers to the 200 mL mark by carefully pouring new test solution down the side of the test containers. Feed 40 *Artemia* nauplii per fish to each test container after the renewal solution has been added.

8.0 TERMINATING THE TOXICITY TEST

After 4 days of exposure final mortality counts are made. Measure temperature, dissolved oxygen, pH, salinity, and ammonia.

9.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and brine controls have acceptable survival. This toxicity test procedure is considered acceptable if topsmelt survival in the dilution control is $\geq 80\%$. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from $20 \pm 1^\circ\text{C}$. Acceptable dissolved oxygen concentration is 60-100% saturation.

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10.0 REFERENCES

US EPA. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. U.S. Environmental Protection Agency, Office of Research and Development. EPA-821-R02-012. October 2002.

11.0 TEST SUMMARY

Species:	<i>Atherinops affinis</i>
Test Duration:	4 Days
Renewals:	48 hours
Organism Source	Aquatic Bio Systems (Fort Collins, CO)
Age of test organisms:	9-14 days
Test Salinity:	Ambient \pm 2‰
Test Temperature:	20 \pm 1°C
Light intensity:	Ambient laboratory illumination 10-20 $\mu\text{E}/\mu^2/\text{s}$
Photoperiod:	16 hour Light: 8 hour Dark
Replication:	5
Test Containers:	1000 mL beakers
Test solution volume:	200 mL minimum
Loading:	5 animals per beaker
Feeding:	40 newly hatched Artemia nauplii per larvae twice daily
Water Quality:	Dissolved oxygen, pH, salinity, ammonia, and temperature
Reference Toxicant:	Zinc sulfate (ZnSO_4)
Daily Monitoring:	Count number alive and remove dead
Acceptability Criteria:	Seawater and Brine Controls: $\geq 80\%$